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PLAGUE-LIKE ORGANISMS IN THE WILD RATS OF SAO PAULO, BRAZIL

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During the study of the prevalence of Leptospira hemorraghiae in the wild rats of the city of São Paulo, a group of interesting organisms were encountered which were very puzzling at first in that they were so similar to the plague bacillus.

It was necessary to catch normal appearing wild rats in various parts of the city, choloroform them, macerate the kidneys in a sterile mortar, with careful aseptic precautions, and inject the resulting emulsion into the peritoneal cavity of guinea-pigs. The work went well up to a certain period, when we began to examine rats that had been caught in the chicken yard of Santa Casa, the large general hospital. One of every three or four of the guinea-pigs that were injected with kidneys of rats caught in this location died within 12 to 24 hours after they were inoculated, though the rats used in the experiments had all appeared normal. The dead guinea-pigs presented all the typical characteristics of animals inoculated with the bacillus of bubonic plague.

This finding was rather alarming, for though there were no known cases of plague in the city, the rats came from the grounds of a hospital at which it was strongly suspected that a recent death had resulted from plague, and the port of Santos, only 40 miles away, was suffering at this time with an outbreak of the disease. Our alarm was not quieted by subsequent study.

Smears from the peritoneal cavity of the dead guinea-pigs showed myriads of gram-negative, bipolar rods, with rounded ends, their morphology being characteristic of the plague bacillus. Pure cultures of the organism were readily obtained from the heart blood of the guinea-pigs.

It was obvious that we were working with an organism which, if it was not the plague bacillus, was certainly closely related to it, and we therefore made an immediate differential study of the organism

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Morphology.—The organism in fresh culture, or when examined directly from animal tissues, was a bipolar bacillus with rounded ends. It was nonmotile, had no capsule and did not form spores. It stained readily with all the common anilin dyes, and was gram-negative. In a 3% sodium chlorid broth it formed involution forms. There was no chain formation, though in broth the bacilli were sometimes seen in twos and fours.

Agar.—The organism grew slowly on agar slants as whitish tiny transparent colonies. At the end of 24 hours the growth was barely visible. The luxuriance of the growth was greatly increased by the addition of a few drops of rabbit blood agar. It grew best at 37 C.; growth took place slowly at 20 C. also, but this is a much less suitable temperature for rapid development than 37 C.

Broth.—The growth was scanty and diffuse even after two weeks' incubation. Good stalactite formation was never obtained in broth cultures though small pellicles formed on the surface of the flask, which had been covered with oil, and extended from 1 to 2 c c into the medium, when the inoculated flasks were placed in a quiet solid place, and allowed to stand for four weeks.

Gelatin.—Growth took place slowly without liquefaction; a faint growth along the needle track was seen only after several days' incubation.

Litmus milk.—There was a faint change in the color of the litmus milk, due possibly to the fermentation of the very small amount, 0.08%, of dextrose-like sugar in milk. There was no coagulation.

Potato.—The growth on potato was invisible. Examination of material from the surface one week after inoculation seemed to indicate that there had been some slight growth.

Hiss' Semisolid Medium.—There was slight acid formation without gas in dextrose, maltose, saccharose and mannite mediums. There was no acid formation in lactose or dextrin. These carbohydrates were the only ones available and the purity of saccharose was not above question.

Sugar Broth Tubes.—Sugar-broth tubes were made and the hydrogen-ion concentration estimated seven days after inoculation. The sugar broth tubes all showed a scanty growth. The same carbohydrates were fermented as in the semisolid mediums. The acid-end point for all tubes in which there was fermentation was about 6.3—estimated by the colorimetric method. The sugar tubes in which no fermentation occurred and also the control uninoculated tubes gave a hydrogen-ion of 7.6.

MacConkey's Sodium Taurocholate Neutral Red Agar.—Repeated inoculations of MacConkey's medium gave negative results. The use of this medium is one of the best methods of differentiation between the plague bacillus and the pasteurellosis group, according to the Indian Plague Commission,[1] because the plague bacillus grows readily on this medium, whereas the other members of the hemorrhagic septicemia group do not.

It is clear therefore that up to this point the chief differential characteristic between the true plague bacillus and the organism we were studying, was that our strain did not grow on MacConkey's medium, and characteristic stalactite formation in broth had not been obtained. This evidence was entirely negative and under the circumstances was far from sufficient. Further confirmation of the cultural findings were urgently needed.

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[1] Jour. Hyg., 1908, 8, p. 302.

Inoculation in Guinea-Pigs.—A very small quantity, 0.005 c c of a 24-hour scanty broth culture inoculated intraperitoneally into a 300 gram guinea-pig always produced death in 14 to 20 hours. There was a wide area of edema and hemorrhage about the point of inoculation, the inguinal and auxiliary nodes were enlarged, deep red in color, with marked hemorrhagic injection about them. The abdomen contained an excess of clear or slightly cloudy serous fluid, with an occasional bit of flaky white fibrin on the surface of the liver. The abdominal vessels were deeply congested. The spleen was enlarged. The pleural cavity contained as a rule 10 to 20 c c of a clear pleural fluid and the lungs were deeply congested. The abdominal and pleural fluid, the liver, and heart blood always contained large numbers of the typical bipolar organism. When the bacilli were inoculated subcutaneously into guinea-pigs in doses of 0.1 to 0.5 c c of a 24-hour broth culture the same pathologic picture was produced as by intraperitoneal inoculation, for a rapid and fatal septicemia ensued within a few hours. A different picture was seen when small doses, 0.005 c c to 0.01 c c, of a 24-hour broth culture were injected subcutaneously, for in these animals an abscess formation usually occurred at the point of inoculation. The animal died on the fifth to seventh day, with extensive fibrinopurulent peritonitis, pleuritis, and pericarditis. In some instances the heart was buried in a coat of fibrin 4 mm. thick. The bipolar organism was found in all the serous cavities and in the heart's blood in considerable numbers, though much less frequently than in the material taken from animals inoculated intraperitoneally.

Broth cultures of the bacillus were rubbed vigorously into the skin of the shaved surface of the abdomen of guinea-pigs, or scratched into the surface of the skin, but in only one instance was infection produced—a slight abscess formation at the point of the scratch, which did not result fatally. Cultures were also rubbed on the nasal mucous membranes, but we were never able to infect by this route. One of the best differential characteristics of the plague bacillus, according to Fritsche,[2] is that infection can be produced in guinea-pigs with the true plague bacillus by simply rubbing the broth culture into the shaved skin of the abdomen, whereas cultures of other plague-like organisms will produce no disease by this method of inoculation.

Rabbits.—The bacillus was very virulent for rabbits—0.005 c c injected intravenously killed an adult rabbit within a few hours (8 to 10) producing all the characteristics of a hemorrhagic septicemia. Intraperitoneal injections of 0.01 c c were rapidly fatal, as was 0.1 c c when injected subcutaneously. The bacilli were always recovered from the heart blood.

White Rats.—White rats were much less susceptible to the bacillus, 0.1 c c of a 24-hour broth culture inoculated intraperitoneally into a half grown white rat, a dose large enough to kill 20 guinea-pigs, produced only moderate symptoms of illness. One half c c of a 24-hour broth culture inoculated subcutaneously did not kill, though a local abscess was formed.

Pigeons.—One of the strains proved pathogenic for pigeons, 0.5 c c of a 24-hour broth culture inoculated intrapectorally producing death in a few hours, and the heart blood was found swarming with bipolar bacilli. This is another differential characteristic of value, for the plague bacillus is never pathogenic to pigeons. This finding does not prove that the organism belonged to the fowl pasteurellosis, for some of the other members of the group are fatal to pigeons.

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[2] Arb. a.d. Kais. Gesundheitsamte, 1902, 18.

Toxin.—The organism was inoculated into 250 c c of plain broth, to which a few drops of rabbit blood was added, and kept at room temperature for 5 weeks. At the end of this time the broth was passed through a Berkefeld filter and the filtrate tested for sterility. Two or three c c of this filtrate contained sufficient toxin to kill a guinea-pig in 24 hours, when inoculated intraperitoneally. This production of toxin is not a differentiating characteristic, for not only the plague bacillus but other members of the hemorrhagic septicemia group also produced a soluble toxin.

Plague Serum.—A small amount of a 24-hour broth culture, 0.05 c c, was mixed with 2 c c of antiplague serum and the mixture injected intraperitoneally. A control animal was injected with the culture only. The animals died 18 to 20 hours later, the control animal succumbing within an hour of the animal inoculated with the mixture of bacteria and serum. This is an additional proof that the organisms were not true plague for the antiplague serum always gives some protection against the plague bacillus.

Agglutination.—Animals were inoculated first with killed and later with living cultures of the organism. Immunity was difficult to produce, and many animals were killed before a serum was obtained which agglutinated the specific strain.

Agglutination for the specific strain occurred in a dilution of 1:10 to 1:20 but no cross agglutination was obtained with any of the strains. Plague serum of a high immunizing titer obtained from the Butantan laboratories did not agglutinate any of the strains.

DISCUSSION

We lost in all six or seven guinea pigs which died from peritoneal infection following inoculation with the kidneys of normal appearing rats which were all captured in the same location. From these guinea-pigs three separate strains of plague-like bacilli were obtained. All three strains were found to be practically identical in regard to their morphologic and cultural characteristics and their virulence to animals, though one strain was virulent to pigeons, while the others were not, and no cross agglutinations were obtained.

It is clear from all the evidence, morphologic, cultural and the results of animal experiments, that we were working with an organism that belonged to the hemorrhagic septicemia group. The only evidence against this was the apparent fermentation of saccharose. We must remember, however, that the hemorrhagic septicemia group consists of a number of organisms, all very similar in morphologic and cultural characteristics and that the plague bacillus is simply the member of this group that produces epidemics of disease in man and rats. The differentiation of the other members of the group from the plague bacillus is usually of more or less academic importance, for the other organisms encountered are usually isolated from frank epidemics in goats, sheep, fowls, horses, hogs, dogs, rabbits, etc., as the case may be.

We had before us a different and intensely practical problem, from the public health point of view. We accidentally found that, in a large city only 40 miles from a port that was at the moment known to be infected with plague, and on the grounds of a hospital where a suspected case of plague had recently come to necropsy, the rats harbored an organism whose morphology was exactly that of the plague bacillus. It is obvious that the burden of proof rested on us to determine accurately and quickly whether or not the organism was the plague bacillus, so that if the results were positive, immediate preventive measures could be taken against an outbreak of plague in man. If we could prove that it was not the plague bacillus but one of the other members of the hemorrhagic septicemia group, then the practical part of the problem was solved. (Before this paper was completed an explosive epidemic of plague did break out in another part of the city).

Let su marshal our evidence against the bacillus being the plague bacillus.

There was nothing about the morphology and staining characteristics that were inconsistent with its being the plague bacillus. In fact, every morphologic requirement for plague was fulfilled.

All the members of the hemorrhagic septicemia group may form stalactites under favorable conditions, so that this is not a good differential characteristic. We were unable to obtain good stalactites even after 5 weeks of growth but this may have been due to unsuitable conditions.

The failure of the organism to grow on maltose sodium taurocholate neutral red medium, though it is negative evidence, is of considerable differential diagnostic importance, since the true plague bacillus grows readily on this medium. The "Indian Plague Commission" considers this medium a valuable differential indicator. It is an item of evidence that needs confirmation, however.

In the first place, it is important to note that all the original rats examined were normal in appearance. This is unusual, to say the least, if the organisms were the plague bacilli, though bacilli of low virulence for rats have been described. Since the organism did not kill white rats even when 0.5 c c of a 24-hour broth culture was inoculated, we must conclude that if the organism was the plague bacillus it had a very low virulence for rats. The pathologic picture produced by intraperitoneal inoculation of small amounts of culture, 0.005 c c, or large amounts subcutaneously, 0.1 c c, into guinea-pigs was characteristic of plague, but the picture produced by the inoculation of small amounts of culture, 0.005 c c subcutaneously into guinea-pigs was quite different from the usual typical picture in plague infected guinea-pigs.

More important evidence was the fact that repeated attempts to infect guinea-pigs by rubbing undiluted 24-hour culture into the shaved skin of the abdomen or into the membranes of the nose were unsuccessful in producing the disease. Fritsche says that true plague will always be produced in guinea-pigs by this method and considers it one of the best differential diagnostic signs. One of the strains proved pathogenic for pigeons, a characteristic that is not found in the true plague bacillus.

From this evidence, therefore, we may reasonably conclude that the organism isolated from the kidneys of normal appearing São Paulo rats belonged to the hemorrhagic septicemia group, but were not plague bacilli. To what group they belonged—fowl, horse, goat, sheep, dog, or rabbit—is more difficult to say, and all are possible since the rats were in intimate contact with all these animals in the Santa Casa yard. The important question in which we were interested was whether or not the disease was plague, and we were able to arrive at a definite conclusion on this point.

Bacillus Enteritidis.—One other organism was encountered in São Paulo rats that resembled the plague bacillus somewhat in its morphologic characteristics. The guinea-pig, which was inoculated with the kidneys of rat 15, died of general peritonitis during the 34th hour. Microscopical examination of the peritoneal fluid showed a pure culture of an organism which was gram-negative and stained bipolarly with methylene blue. Cultures from the heart blood were made on agar slants and incubated. The following morning the agar slant was covered with a diffuse moist whitish growth. This characteristic of rapid growth was alone almost sufficient to rule out the possibility that the organism was the plague bacillus. Subsequent study rapidly classified the organism as belonging to the B. enteritidis group. Bates[8] found a similar "safety pin"

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[3] Proc. Canal Zone Med. Assn., 1916, 7, p. 61.

organism in an enlarged gland of a rat examined at necropsy in the Canal Zone, which he identified as belonging to B. enteritidis group, and numerous epizootics have been recorded in rats and rabbits, due to the gaertner bacillus. According to some authors, the gaertner bacillus is a normal inhabitant of the rat's intestine. It is one of the easiest bipolar organisms to differentiate from the plague bacillus because of its rapid growth on all ordinary mediums, with brownish growth on potato, and its motility.

CONCLUSIONS

Three strains of bacilli were isolated from normal appearing rats of São Paulo, at a time when the city was in close proximity to and in constant danger from bubonic plague. It was proved by cultural methods and animal inoculation that these organisms were not plague but belonged to the closely allied pasteurellosis group.

A rapid and sure diagnosis of B. pestis is not always a simple matter. One is not justified in relying on one or two modes of differentiation only, but one must apply every differential method known.

B. enteritidis, which may resemble B. pestis somewhat in morphologic and staining characteristics, was isolated from the kidneys of a normal appearing rat. The differentiation between B. pestis and B. enteritidis is a simple and rapid process.